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Control of bitter rot and blue mold of apples by integrating heat and antagonist treatments on 1-MCP treated fruit stored under controlled atmosphere conditions

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Abstract

To maximize control of fruit decay by alternatives to synthetic fungicides after harvest, various control strategies can be integrated. Treatment of fruit with antagonists is one of the most promising alternatives. This treatment, however, has little or no eradicative activity, which limits its use. Fruit treatment with hot air (at 38 °C) for 4 d has eradicative but no residual activity against blue mold (caused by *Penicillium expansum*) on apple, and 1-methylcyclopropene (1-MCP) is an ethylene receptor inhibitor which slows apple maturation and, presumably, extends action of natural defense mechanisms. An antagonist, *Metchnikowia pulcherrima* T5-A2, was used in combination with heat and 1-MCP treatments to control bitter rot (caused by *Colletotrichum acutatum*) and blue mold (caused by *P. expansum*) on 'Golden Delicious' apples under controlled atmosphere (CA) conditions. 1-MCP treatment increased bitter rot and blue mold decays, but both of these decays were effectively controlled on 1-MCP treated apples by a combination of the antagonist and heat treatments. *C. acutatum* is a weaker pathogen than *P. expansum*, and bitter rot, even on the control treatments, developed only after 4 months in CA storage followed by 2 weeks incubation at 24 °C. In contrast, non-treated fruit inoculated with *P. expansum* were completely decayed after 2 months in CA. The antagonist controlled bitter rot more effectively than blue mold, while blue mold was more effectively controlled by heat treatment. The use of 1-MCP on harvested fruit to inhibit maturation can predispose fruit to decay, but the alternatives to synthetic fungicides are capable of preventing this increase in decay.
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Keywords: 1-Methylcyclopropene; *Penicillium expansum*; *Colletotrichum acutatum*

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1. Introduction

Fungicides have been the main control method for reducing postharvest decays on fruits for the past few decades. However, increasing restrictions on the use of fungicides (Gullino and Kuijpers, 1994; Ragsdale and Sisler, 1994), due to the perceived harmful effects on human health and environment, and the declining effectiveness of many fungicides due to the development of resistance in postharvest pathogens, necessitates the development of alternative control methods. For some fruits there are no fungicides registered for postharvest use, but significant losses from postharvest decays occur. Biological control of postharvest diseases (BCPD) has emerged recently as a very effective alternative. Effective antagonists have been found against major postharvest pathogens of pome, stone, citrus and subtropical and tropical fruits worldwide (Janisiewicz and Korsten, 2002). The first two products for controlling postharvest diseases of pome and citrus fruits, BioSave (EcoScience Corp., Orlando, FL) and Aspire (Ecogen Inc., Langhorne, PA), were introduced commercially in the United States in 1996. The fruit industry proved to be flexible in adopting this new technology, and the use of biocontrol has been increasing steadily. For example, the use of BioSave products that are based on strains of *P. syringae* not only increased on pome and citrus fruits, but the label has been expanded to include cherries and potatoes, and further use on other fruits and vegetables looks promising (Lucie Grant, EcoScience Corp., personal communication). BCPD has its limitations and can not be used under all circumstances. The major limitations are lack of eradicative activity and a more limited spectrum of activity than synthetic fungicides. Various alternatives to fungicide treatment developed during the past few decades have never been commercially implemented or were implemented only on a limited scale because of insufficient decay control. Some of these methods, such as heat treatment (4 d at 38 °C) (Conway et al., 1999; Leverentz et al., 2000), and use of substances generally regarded as safe such as calcium chloride (Janisiewicz et al., 1998; McLaughlin et al., 1990; Wisniewski et al., 1995), diluted ethanol (Mari and Carati, 1998), chitosan (El-Ghaouth et al., 2000; Mari and Carati, 1998), or carbonate salts (Mari and Carati, 1998; Smilanick et al., 1999) when combined with biocontrol agents, resulted in additive or synergistic effects and addressed the major limitation of biological control. For example, combining calcium chloride treatment with the antagonist *P. syringae* strain ESC 11 resulted in better control of blue mold (caused by *Penicillium expansum*) than either treatment alone (Janisiewicz et al., 1998). The beneficial effect of calcium was most apparent after 3 and 6 months in storage, when the fruit had matured and the effectiveness of biocontrol declined.

There has been an increasing interest in the use of pre-storage heat treatments to control insect pests, prevent fungal decay, and modify ripening of commodities (Lurie, 1998). Heat treatment (hot air at 38 °C for 4 d) is effective in controlling blue mold on apples, but has no residual activity (Falik et al., 1995; Lurie et al., 1995). Combining hot air (4 d 38 °C) treatment with heat tolerant antagonists also resulted in better control than either treatment alone (Conway et al., 1999; Leverentz et al., 2000). These treatments complemented each other in that heat provided eradicative activity up to 24 h after inoculation with *P. expansum*, and biocontrol agents provided residual activity.

Treatment of preclimacteric apples with the ethylene action inhibitor, 1-methylcyclopropene (1-MCP), delays fruit ripening (Baritelle et al., 2001; Fan et al., 1999; Fan and Mattheis, 1999; Rupasinghe et al., 2000; Watkins et al., 2000) In non-climacteric fruit, 1-MCP can increase, decrease or have no effect on pathogen-induced decay development (Ku et al., 1999; Mullins et al., 2000; Porat et al., 1999).

Bitter rot (caused by *Colletotrichum acutatum*) is a potentially destructive disease in regions east of the Great Plains in the United States (Pierson et al., 1971). Decay caused by this pathogen often develops in the orchard close to harvest, or in storage from incipient infection in the orchard (Anderson, 1956; Pierson et al., 1971). However, in hot and humid years when inoculum levels are high, drenching fruit before storage may result in cross-contamination of wounded fruit with the spores from decaying fruit, resulting in severe

decay of the wounded fruits (Janisiewicz, personal observation).

The ultimate goal of our research is to maximize control of apple decay by combining various alternatives with biological control to address the limitation of biocontrol. The specific objective of this research was to determine the effect of 1-MCP, heat and antagonist treatments on bitter rot and blue mold on 'Golden Delicious' apple stored under controlled atmosphere (CA) conditions. We used a heat tolerant (4 d at 38 °C) yeast, *Metchnikowia pulcherrima* strain T5-A2, isolated from wounded apple in an unmanaged orchard. This yeast is a common inhabitant of fruit including apple and grape (Bowen and Beech, 1964; Davenport, 1976). It is commonly found in apple cider and is an integral part in the wine-making process (Beech, 1993; Bowen and Beech, 1964; Chamberline et al., 1997; Schuetz and Gafner, 1993).

2. Materials and methods

2.1. Fruit

'Golden Delicious' apples were harvested at the preclimacteric stage [ethylene production <2 pmol kg^{-1} s^{-1}] from a commercial orchard in Pennsylvania. The climacteric rise in CO_2 production had not yet begun (~ 50 nmol kg^{-1} s^{-1})]. The apples were randomized prior to treatment.

2.1.1. Assessment of fruit quality

Respiration and ethylene production rates of non-treated control and 1-MCP-, heat- and 1-MCP and heat-treated fruit were monitored every 8 h during a 7-d period using an automated system (Izumi et al., 1996). Four five-fruit replications were measured after 0, 2 and 4 months in CA storage at 1 °C. The starch content of non-treated fruit at harvest was measured using the Cornell generic starch scale of 1–8 (Blanpeid and Silsby, 1992).

Peel color, Magness-Taylor (MT) firmness, soluble solids content (SSC), and titratable acidity (TA) were done on the same 20-fruit lots of fruit after 0, 2 and 4 months in CA storage at 1 °C plus 1 d or 2 weeks at 20 °C in air following the 2- and 4-month CA storage, respectively. The color (L*, a*, and b*) of the apple peel at two non-blushed sites of each fruit was measured using a chroma meter (model CR-300, Minolta, Tokyo, Japan). A decrease in L* indicated a loss of whiteness (brightness), and a more positive a* value indicated degreening had occurred, whereas a more positive b* indicated yellowing. Firmness was measured with an electronic fruit firmness tester (model EPT-1, Lake City Technical Products, Kelowna, BC, Canada) set in the MT mode and interfaced with a personal computer. MT firmness was measured at two opposite points on the equator of each fruit after removing a thin slice of peel from each site. Both SSC and TA were determined using freshly prepared juice. Individual fruit were ground in an electric juice extractor. The SSC was measured using a digital temperature-compensated refractometer (model PR-101, Atago Co. Tokyo, Japan), and TA, expressed as malic acid, was determined by titrating 10-ml juice with 1.0 M KOH to pH 8.2 (Mitcham and Kader, 1996).

2.2. Pathogens

The *P. expansum* (MD-8) isolate used was a very aggressive one from our collection, which had been previously isolated from a decayed apple in storage and was used in various biocontrol tests (Janisiewicz and Jeffers, 1997; Janisiewicz and Marchi, 1992). The *C. acutatum* isolate was obtained from Kenneth D. Hickey, Penn State Fruit Research Laboratory and Extension Center, Biglerville, PA. Both fungi were maintained on potato–dextrose–agar (PDA) and continued virulence was assured by periodic transfers through apple. The conidial suspensions (1×10^4 conidia ml^{-1}) used for fruit inoculations were prepared from 10-day-old cultures as previously described (Janisiewicz and Marchi, 1992).

2.3. Antagonist

The heat tolerant antagonist used was the yeast *M. pulcherrima* strain T5-A2 isolated from wounded apples and was capable of growing at cold temperatures (Janisiewicz et al., 2001). The

yeast was grown in 50 ml of nutrient yeast–dextrose–broth medium in 250-ml Erlenmeyer flasks on a rotary shaker at 150 r.p.m. at 26 °C. Following incubation for 24 h, the cells were harvested by centrifugation at $7000 \times g$ for 10 min, resuspended in water, and the concentration adjusted to 3×10^7 CFU ml^{-1} with a spectrophotometer at 420 nm.

2.4. Heat tolerance of the pathogens

Conidia from 12-day-old cultures of *P. expansum* and *C. acutatum*, grown on PDA in 10 cm diameter Petri plates, were harvested using a Pasteur pipet and placed in 5 cm diameter Petri plates. The open plates were placed in a desiccator with silica gel packets lining the bottom of the desiccator and incubated for 40 d at 22 °C. The desiccator was then opened, lids were placed on the Petri plates, sealed with parafilm, and five plates were incubated at room temperature (controls) and five plates at 38 °C. The plates were removed at daily intervals (one plate each day), conidia were suspended in several drops of 0.05% Tween 80, and the concentration was adjusted to 1×10^6 conidia ml^{-1} in water using a hemacytometer. Aliquots of 100 μl of the resulting suspensions were plated on PDA plates. Fungal colonies were marked and counted after 2 d incubation at 22 °C, and plates were rechecked for possible newly developing colonies for two more days.

2.5. Fruit inoculation and treatments

2.5.1. Fruit inoculation

The fruit were wounded with a six-penny nail to a depth of 3 mm, and the wounds were inoculated with 25 μl per wound of either *P. expansum* or *C. acutatum* conidia alone, or the individual pathogens combined with the antagonist. Immediately after inoculation (0 h), one set of inoculated apples was either not treated (moved to cold storage) or subjected to treatments with 1-MCP, heat, or 1-MCP+heat. The other set was similarly treated after 12 h incubation at an ambient temperature of ~24 °C. There were three replications of 15 fruit per treatment. All of the fruit were then placed in CA storage in a completely randomized design and evaluated for decay development after 2 and 4 months, and again after an additional 2 weeks at ambient temperature following removal after 4 months. Non-treated (control) fruit that were severely decayed by *P. expansum* after 2 months in storage were discarded after evaluation.

2.5.2. 1-MCP treatment

After inoculation, apples designated for 1-MCP treatment were separated into lots of 122 fruit, placed into plastic mesh bags, and sealed in 208-l stainless steel containers containing 200 g soda lime to absorb CO_2 and a fan. A stock 1% concentration of 1-MCP was prepared by mixing 800 mg SmartFresh (Rohm and Haas, Philadelphia, PA) with 10 ml water in a 60-ml syringe at room temperature. Stock 1-MCP was injected as needed to maintain the 1-MCP concentration within the chambers between 0.4 and 0.5 μl l^{-1} for 17 h at 20 C. Previous experiments indicated that a 1-MCP treatment of 0.3 μl l^{-1} for 17 h at 20 °C essentially prevented climacteric ethylene production and thereby ethylene-mediated ripening. Following 1-MCP treatment and before boxing, apples were placed on trays and air equilibrated for 9 h to allow for 1-MCP outgassing from the fruit. Chamber gas samples were collected five times during the treatment period and measured for 1-MCP levels using a gas chromatograph (model 5890 Series II, Hewlett Packard, Rockville, MD) fitted with a Haycep Q column (3 m × 3 mm) and a photoionization detector. The carrier gas was ultra purified helium at a flow rate of 2 ml min^{-1}. The temperature program was isothermal for 1 min at 35 °C and then raised at the rate of 20 °C min^{-1} to 90 °C and held for 3 min. The calibration gas for 1-MCP analyses was 1.05 μl l^{-1} isobutylene in air.

2.5.3. Heat treatment

Inoculated apples, treated or not treated with 1-MCP, and designated for heat treatment, were placed in tray-packed boxes with perforated polyethylene bags as liners and then either heated immediately or stored at 24 °C for 12 h and then heated in a thermostatically controlled (±1 °C) walk-in chamber. Apples were heated at 38 °C for

4 d and the relative humidity was maintained at > 85% in the chamber. Storage conditions were monitored with a hygrothermograph. The non-heated apples were placed at 1 °C for the same time period. All fruit were removed from heat or cold storage after 4 d and moved to CA storage at 0.5 °C.

2.5.4. Controlled atmosphere storage

The fruit were stored in two 1.2 m^3 units of six barrels (0.2 m^3 per barrel) at 0.5 °C under CA conditions of 1.1% oxygen and 1.8% carbon dioxide. CA storage conditions were measured four times each day and adjustments were made by a David Bishop Instruments Oxystat 2002 controller (David Bishop, Heathfield, UK). Oxygen and carbon dioxide levels were measured by paramagnetic and infrared sensors, respectively. The relative humidity within each CA system was > 90%.

2.6. Antagonist recovery

The populations of the *M. pulcherrima* T5-A2 in the wounds were determined immediately after inoculation and just after the treatments (Time 0), after 2 and 4 months in CA storage, and after 4 months in CA storage, followed by 2 weeks at 20 °C. The antagonist was recovered from the wounds of four apples per treatment, except the last sampling time, at which only three apples per treatment were used. The recovery, plating, incubation and counting of the colonies was performed according to the procedure described previously (Conway et al., 2000).

2.7. Statistical analyses

The lesion severity data were analyzed by ANOVA (analysis of variance) using PROC MIXED (SAS Inst.) With Treatments as the fixed effect and Tray as the random effect. The assumptions of the mixed linear model were checked. Variance grouping was used to correct for variance heterogeneity. The mean comparisons were done with Sidak adjusted *P*-values so that the experiment-wise error was 0.05.

Data from *C. acutatum* inoculated apples were taken only after 4 months in storage at 0.5 °C + 2 weeks at 24 °C. All four treatments that were inoculated with the antagonist, but not treated with 1-MCP, had apples with no or few lesions. These data were not included in the statistical analysis. Data from *P. expansum* inoculated apples treated with heat were log $(n+1)$ transformed to correct for non-normality.

The recovery data of *M. pulcherrima* T5-A2 populations from apple wounds were analyzed by ANOVA as a four factor linear model using PROC MIXED (SAS Inst.) with Time, Incubation, 1-MCP and Heat as the factors. The assumptions of the general linear model were checked. To correct for non-normality the recovery values were $\log_{10}(x)$ transformed. Variance grouping was used to correct for variance heterogeneity.

3. Results

3.1. Heat tolerance of the pathogens

The viability of *C. acutatum* conidia declined by two log units during the 40 d drying period in the desiccator at 22 °C, while *P. expansum* conidia remained unaffected (Fig. 1, time 0). Incubating dry conidia of *P. expansum* at 38 °C caused a decline in their viability by 4.5 log units during 96 h. Conidia in the control treatment, kept at 22 °C, declined less than 1 log unit during that period. *C. acutatum* conidia were not affected by exposure to 38 °C during 96 h, and their viability was comparable to those kept at 22 °C.

3.2. Decay severity

Decay severity was determined by measuring the lesion diameters at each evaluation period. Almost no decay developed on *C. acutatum* inoculated fruit after 2 and 4 months in CA storage at 0.5 °C regardless of the treatment. Therefore, fruit removed from storage after 4 months were incubated for an additional 2 weeks at room temperature before the final decay measurements. Only the final data were subjected to the statistical analysis. Analysis of variance using PROC MIXED (SAS

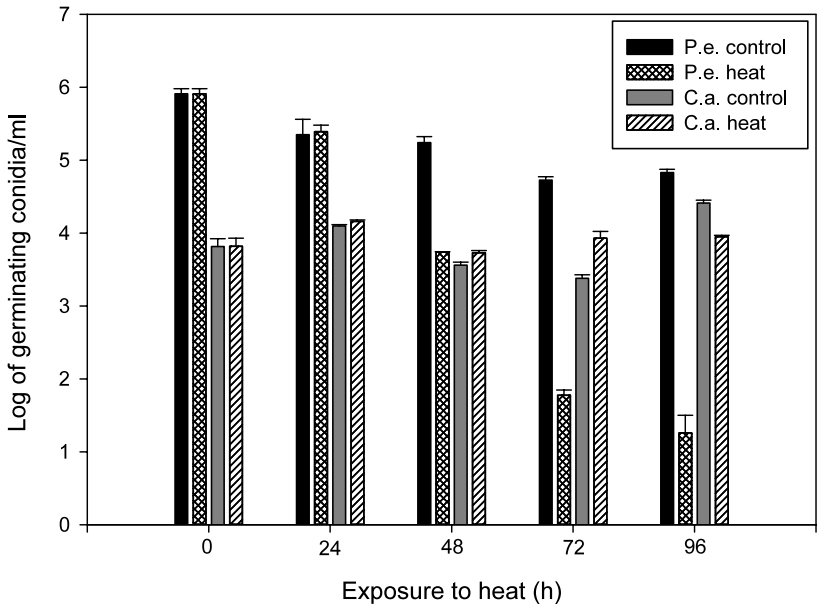


Fig. 1. Germination of conidia of *C. acutatum* and *P. expansum* on PDA medium after drying for 40 d in a desiccator and exposure to 38 °C (heat) for various periods of time. Bars represent ± standard deviation of the means.

Inst.) revealed that the main effects antagonist and initial incubation after inoculation (0 or 12 h), and the interaction of antagonist × 1-MCP were highly significant (P-value < 0.0001). The mean lesion sizes were 4.6 and 12.5 mm on antagonist treated and non-treated fruits, respectively, and 8.3 and 12.4 mm on fruit incubated after inoculation for 0 and 12 h (ambient temperature), respectively. Treatment with 1-MCP resulted in increased lesion size over fruit not treated with 1-MCP, but the lesions were smaller on fruit treated with the antagonist in addition to 1-MCP (Table 1, Fig. 2). In general, heat treatment did not reduce lesion severity on *C. acutatum* inoculated fruit (Table 1).

After 2 months in storage, large lesions developed on non-treated *P. expansum* inoculated (control) fruit and the average lesion size ranged from 35.1 mm (0 h) to 51.7 mm (12 h + 1-MCP) (Fig. 3). These fruit were discarded after evaluation. The average lesion size for non-heated fruit that were treated with the antagonist ranged in size from 9.0 mm (12 h) to 14.9 mm (12 h + 1-MCP). Treatments that included heat had no lesions, except for a few small lesions in two treatments without the antagonist on 1-MCP treated fruit, regardless of incubation period. These treatments were excluded from the statistical analysis. The data from the non-heated fruit stored for 2 months were analyzed using PROC MIXED (SAS Inst.). Antagonist, initial incubation (0 or 12 h), 1-MCP, and antagonist × incubation, and antagonist × 1-MCP interactions were significant. Overall, on the non-heated fruit, application of the antagonist reduced average lesion size from 42.9 to 12.4 mm. The longer incubation time resulted in an increased lesion size from 26.3 mm (0 h) to 28.9 mm (12 h), and 1-MCP treatment caused an increase in the lesion size from 23.8 to 31.5 mm. The longer incubation (12 h) and 1-MCP treatment resulted in an increase in lesion size on the non-antagonist treated fruit, and on antagonist treated fruit in the case of 1-MCP treatment (Table 2).

After 4 months in storage, fruit not treated with heat had developed large lesions and were not included in the analysis (Fig. 3). Treatments with fruit that were heated had no or very small lesions and were incubated for an additional 2 weeks at ambient temperature. The data from the last measurement were analyzed as a four factor mixed

Table 1
Two-way interaction means of the lesion severity (lesion diameter in mm) data from *C. acutatum* inoculated apples after 4 months at 0.5 °C+2 weeks at 24 °C storage

Inoculation	Interaction means					
	Incubation		1-MCP		Heat	
	0 h	12 h	No	Yes	No	Yes
C. acutatum	10.70 a[a]y[b]	14.35 ax	10.37 y	14.67 ax	12.16 ax	12.88 ax
C. acutatum +Antagonist	3.61 bx	5.50 bx	n/a[c]	4.56 b	5.86 bx	3.26 bx

[a] Means within columns Incubation/1-MCP/Heat with different letters (a and b) are significantly different ($P = 0.05$).
[b] Means within rows Incubation/1-MCP/Heat with different letters (x and y) are significantly different ($P = 0.05$).
[c] Not used in the analysis; little or no lesions developed.

linear model using PROC MIXED (SAS Inst.). Antagonist, antagonist × incubation, and antagonist × 1-MCP interactions were significant (*P*-value < 0.01). Overall, the antagonist reduced lesion size from 2.2 to 0.5 mm. Antagonist application reduced lesion size on fruit incubated

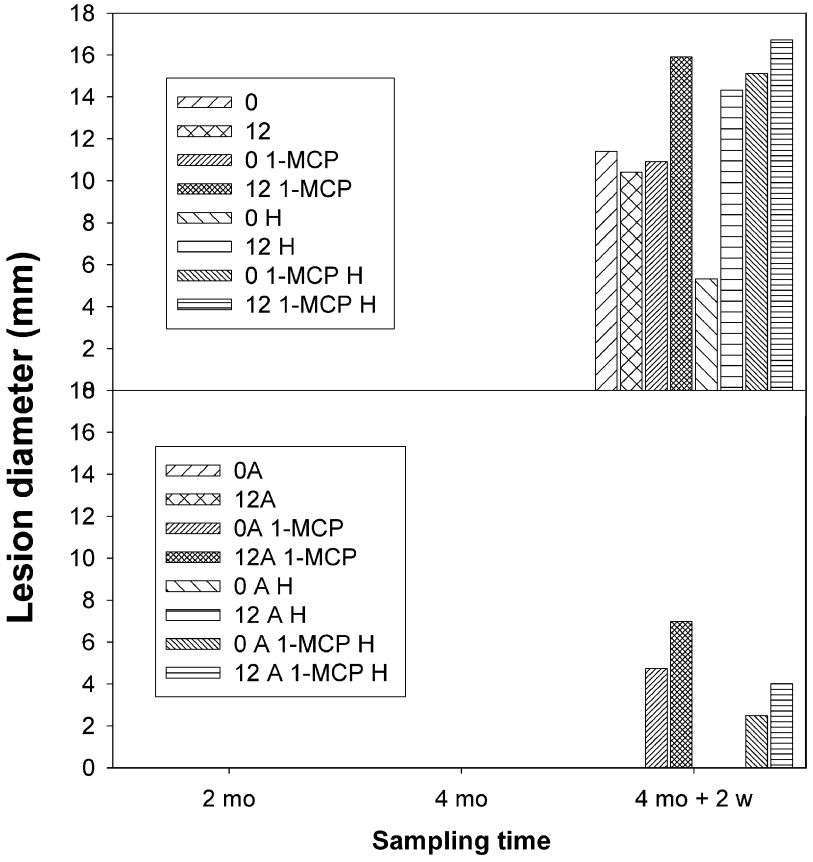


Fig. 2. Decay severity on 'Golden Delicious' apples inoculated with *C. acutatum*, subjected to various treatments (0, 12 = hours of incubation at room temperature after inoculation; A, antagonist; H, heat; 1-MCP) and stored at 0.5 °C under CA conditions for various periods of time.

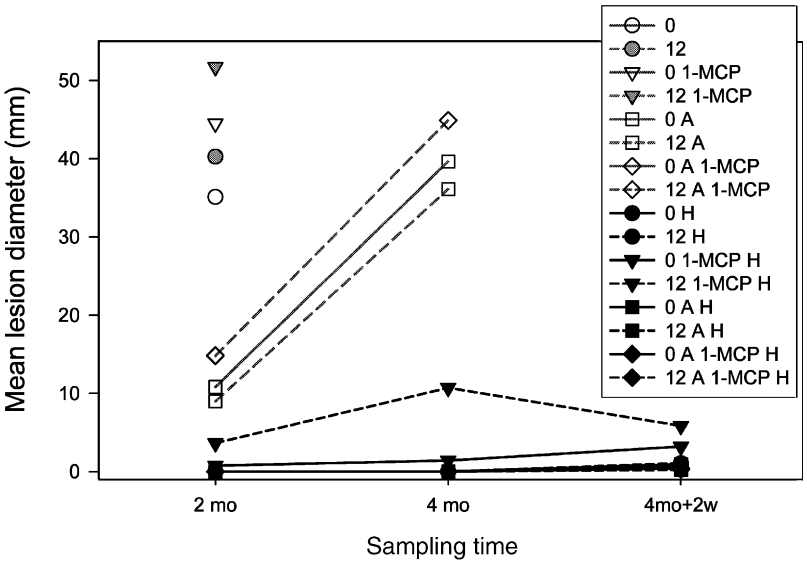


Fig. 3. Decay severity on 'Golden Delicious' apples inoculated with *P. expansum*, subjected to various treatments (0, 12 = hours of incubation at room temperature after inoculation; A, antagonist; H, heat; 1-MCP) and stored at 0.5 °C under CA conditions for various periods of time.

for 12 h but not on those incubated for 0 h. Treatment with 1-MCP resulted in an increased lesion size on fruit not treated with the antagonist, but not on fruit treated with the antagonist (Table 2). On heat treated fruit, the average lesion did not exceed 5.9 mm in any of the treatments (Fig. 3). In one treatment (12 h- 1-MCP- heat), the mean values declined after 4 months+2 weeks storage, because some fruit had decayed rapidly during the last 2 weeks and could not be included in the final analysis of lesion severity and incidence (Fig. 3).

3.3. Decay incidence

To determine the incidence of the decay for the fruit inoculated with *C. acutatum* or *P. expansum*,

Table 2
Two-way interaction means of the lesion severity (lesion diameter measured in millimeter) from *P. expansum* inoculated apples that were heated or non-heated and stored for 2 or 4 months at 0.5 °C

		Interaction means			
		Incubation		1-MCP	
	Inoculation	0 h	12 h	No	Yes
Non-heated	2 Months				
	P. expansum	39.80 a[a] y[b]	45.99 ax	37.69 ay	48.10 ax
	P. expansum+Antagonist	12.84 bx	11.89 bx	9.90 by	14.83 bx
Heated	4 Months+2 weeks at 24 °C.				
	P. expansum	1.64 a[a]x[b]	2.78 ax	0.86 ay	4.37 ax
	P. expansum+Antagonist	0.67 ax	0.27 bx	0.56 ax	0.36 bx

[a] Means within columns for each storage time (2 or 4 months+2 weeks storage) with different letters (a and b) are significantly different ($P = 0.05$).

[b] Means within rows for Incubation or 1-MCP treatment with different letters (x and y) are significantly different ($P = 0.05$).

the number of apples without lesions was determined for each treatment. A χ^2-analysis of the 16 treatments inoculated with *C. acutatum* (Table 3) showed that the frequency distributions were not all the same ($\chi^2 = 132.2$, P-value ≤ 0.0005). Analysis of the best four treatments (lowest incidence of decay) showed that they were not statistically different ($\chi^2 = 0.1416$, P-value ≤ 1.000). None of the top four treatments included 1-MCP treatment and all were treated with the antagonist. All of the worst four treatments (highest incidence of decay) were 1-MCP treated and none were treated with the antagonist. All antagonist treated fruit had fewer lesions than non-antagonist treated fruit, with one exception (12 h incubated, 1-MCP treated, no heat).

The percentage of fruit with *P. expansum* decay was calculated for each evaluation time (Fig. 4). In one treatment, the 12 h- 1-MCP- heat-treatment, the mean values declined after 4 months + 2 weeks storage, because some fruit had decayed rapidly during the last 2 weeks and could not be included in the final analysis of lesion severity and incidence. χ^2-analysis of the 12 treatments inoculated with *P. expansum*, which excluded treatments where all fruit were infected, showed that the frequency distributions were not all the same ($\chi^2 = 33.11$, P-value ≥ 0.0538). Further analysis of the three most effective treatments (lowest incidence of decay) showed that they were not statistically different ($\chi^2 = 0.0138$, P-value $\geq$ 1.000). All of these treatments included the antagonist and heat. All eight of the most effective treatments included heat treatment, while the four least effective treatments included neither the antagonist nor heat.

3.4. Antagonist recovery

Analysis of the recovery data of *M. pulcherrima* T5-A2 populations from apple wounds showed a significant four-way interaction of Heat × 1-MCP × Incubation Time × Sampling Time. Plots of the four-way interaction means, i.e. the treatment means, indicate that this is largely due to the different behavior over time between the heat and non-heat treatments (Fig. 5). The comparisons of the means were conducted with Sidak adjusted P-values so that the experiment-wise error was 0.05. The antagonist populations increased as much as 0.5 log units during 12 h incubation and application of various treatments at time 0 at ambient

Table 3
Number of apples without decay (out of 45 per treatment) at various sampling times on fruit inoculated with *C. acutatum* and subjected to various treatments.

Treatment				Sampling time		
Antagonist	Incubation (h)	1-MCP	Heat	2 Months	4 Months	4 Months + 2 weeks
y[1]	12	n	y	45	45	44
y	12	n	n	45	45	41
y	0	n	n	45	45	40
y	0	n	y	43	43	40
y	0	y	y	43	43	33
y	12	y	y	45	45	32
y	0	y	n	45	45	28
n	0	n	y	45	45	24
n	0	n	n	44	42	14
y	12	y	n	45	45	13
n	12	n	n	45	44	10
n	12	n	y	45	45	8
n	0	y	n	45	45	7
n	0	y	y	45	44	5
n	12	y	n	45	45	2
n	12	y	y	41	38	2

[1] y, treatment was applied; n, treatment was not applied.

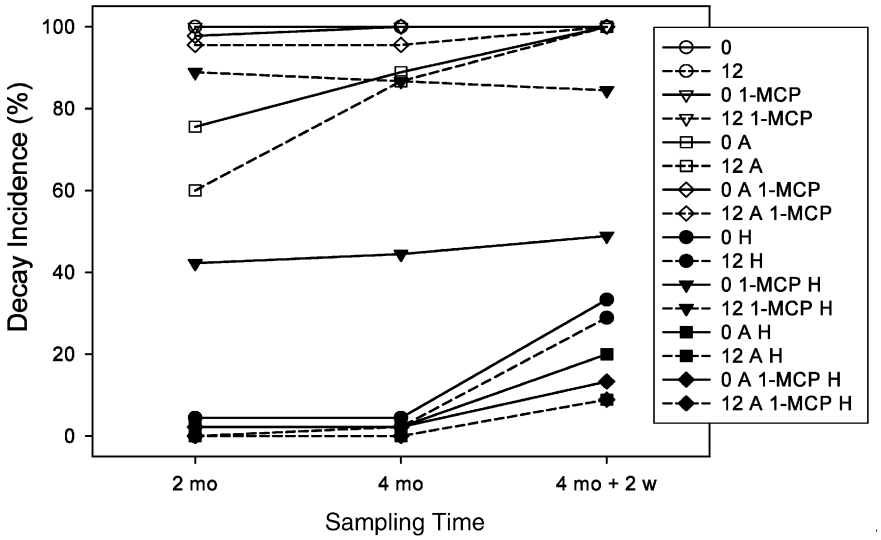


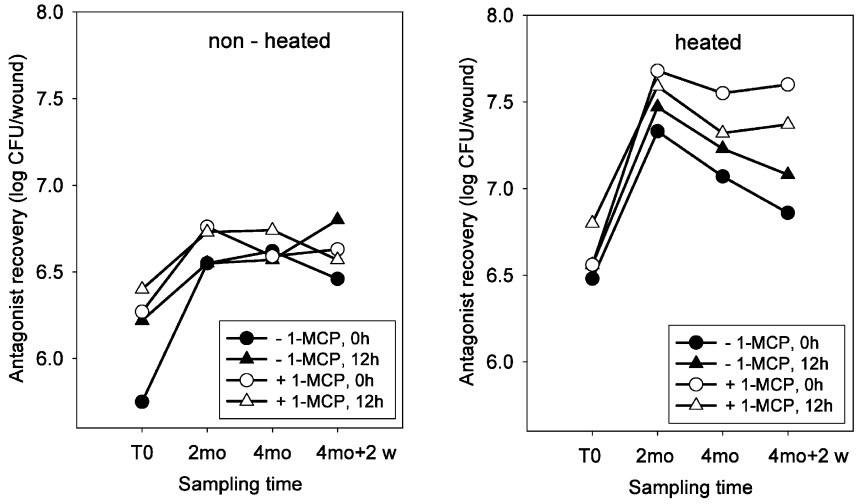
Fig. 4. Decay incidence on 'Golden Delicious' apples inoculated with *P. expansum*, subjected to various treatments (0, 12 = hours of incubation at room temperature after inoculation; A, antagonist; H, heat; 1-MCP), and stored at 0.5 °C under CA conditions for various periods of time.

temperature (Fig. 5). Populations on fruit incubated for 12 h and treated with 1-MCP and heat were significantly higher than in other treatments on heated apples at time 0. During storage, the populations continued to increase and appeared to stabilize after 2 months. The populations on heated fruit were between 0.7 and 0.9 log units higher than on non-heated fruit. During the next 2 months of storage, populations on non-heated fruit were stable while those on heated fruit appeared to decline. This decline continued during the additional 2 weeks of storage at room temperature on non-1-MCP treated but not on 1-MCP treated fruit.

3.5. Fruit physiology

Following harvest but prior to treatment with 1-MCP and/or heat, the fruit entered the climacteric stage of development, as indicated by rapid increases in the respiration (from $\sim$50 to 90.4 nmol kg^{-1} s^{-1}) and ethylene production (from $<$ 2 to 4.1 pmol kg^{-1} s^{-1}) rates. Both rates are about double the corresponding rates observed in preclimacteric 'Golden Delicious' fruit. At harvest, the starch index was 5.6 using the Cornell generic starch chart scale 1–8, and firmness was $<$85 N, further suggesting that the fruit were mature. Within 7 d after harvest, the respiration and ethylene production rates in non-treated fruit held at 20 °C had stabilized at 148.8 nmol kg^{-1} s^{-1} and 1382 pmol kg^{-1} s^{-1}, respectively (Table 4). The heat treatment initially inhibited the climacteric rise in respiration and ethylene production rates, whereas the 1-MCP treatment and 1-MCP plus heat treatment decreased the respiration and ethylene production rates to preclimacteric levels. None of the treatments had any immediate impact on firmness, peel color or SSC content, but heat and 1-MCP plus heat treatments decreased TA.

The 1-MCP treatment prevented the climacteric rise in respiration and ethylene production rates throughout storage. 1-MCP also maintained firmness, TA and peel color throughout storage. Throughout the storage period, the heat treatment had little inhibitory effect on respiration and ethylene production rates, and only inhibited softening up to 2 months. However, heat increased peel degreening (less negative a* value) and acidity loss during storage. 1-MCP plus heat treatment delayed, but did not prevent, the onset of climacteric respiration and ethylene production, and peel degreening. None of the treatments affected SSC



Treatment		Non-heated apples					Heated apples			
Inc (h)	1-MCP	0	2 mo	4 mo	4 mo+2w		0	2 mo	4 mo	4mo+2w
0	n	5.75 b[1]	6.55 a	6.62 a	6.46 a		6.48 b[1]	7.33 c	7.07 b	6.86 c
12	n	6.22 a	6.55 a	6.57 a	6.80 a		6.56 b	7.47 bc	7.23 b	7.08 bc
0	y	6.27 a	6.76 a	6.59 a	6.63 a		6.56 b	7.68 a	7.55 a	7.60 a
12	y	6.40 a	6.73 a	6.74 a	6.57 a		6.80 a	7.59 ab	7.32 ab	7.37 b

[1] Means with different letters within columns are significantly different (P= 0.05) according to Sidak adjusted p-values.

Fig. 5. Recovery of *M. pulcherima* T5-A2 from non-heated and heated 'Golden Delicious' apples subjected to various treatments and stored at 0.5 °C under CA conditions for various periods of time.

of the fruit during storage, although large fruit-to-fruit variations in SSC may have precluded detection of minor treatment effects.

4. Discussion

Since little or no bitter rot developed on *C. acutatum* inoculated apples, as compared to extensive blue mold decay on *P. expansum* inoculated non-heated apples after 4 months in CA cold storage, the fruit were held for 2 weeks at 24 °C to enhance the probability of bitter rot development. This caused greater development of bitter rot and blue mold, and facilitated determination of the effectiveness of the various treatments. Although the heat treatment had little effect on reduction of bitter rot, *M. pulcherrima* T5-A2 treatment effectively controlled this decay. These results support our preliminary tests in which this antagonist had been effective in controlling bitter rot (Janisiewicz, unpublished), and the in vitro tests where the pathogen was resistant to the heat treatment. The bitter rot decay became visible after the apples had been taken out of cold storage and stored at room temperature for 2 weeks. However, the presence of the antagonist was able to keep this decay development under control. The lack of lesion development after 4 months in CA storage may have resulted from either fruit still being resistant to the pathogen, CA storage conditions, or the low temperature (0.5 °C) regime, which prevented decay development. These possibilities are currently being investigated.

Extensive blue mold development on *P. expansum* inoculated non-heated apples indicates that this pathogen is more aggressive than *C. acutatum* under these storage conditions. Reduction of the

Table 4
Maturity indices of 'Golden Delicious' apples subjected to various treatments and stored in CA storage at 0.5 °C for 0, 2, and 4 months

Treatment	Respiration [nmol $kg^{-1} s^{-1}$]	Ethylene production [pmol $kg^{-1} s^{-1}$]	Firmnes s [N]	SSC [Brix]	TA [% malic acid]	Peel color			Starch index
						L*	a*	b*	
0 Month									
Non-treated	148.8 a[a]	1382 a	81.6 a	13.5 a	0.47 a	76.6 a	−14.0 a	40.3 a	5.6
1-MCP	43.8 c	2 c	81.0 a	13.3 a	0.47 a	76.5 a	−13.8 a	40.3 a	
Heat	113.3 b	1001 b	80.9 a	14.7 a	0.39 c	76.9 a	−11.3 a	41.0 a	
1-MCP+Heat	53.8 c	4 c	80.7 a	14.5 a	0.42 b	76.0 a	−12.1 a	42.8 a	
2 Months + 1 dat 20 °C									
Non-treated	161.5 a	1531 a	68.9 b	15.0 a	0.41 ab	77.3 ab	−13.0 a	41.9 a	
1-MCP	46.4 b	10 b	77.0 a	15.3 a	0.48 a	75.7 b	−13.6 a	42.1 a	
Heat	156.4 a	1501 a	75.2 a	16.1 a	0.36 b	78.3 a	−9.9 b	43.7 a	
1-MCP+Heat	54.6 b	31 b	75.9 a	15.4 a	0.33 b	76.5 ab	−11.4 ab	43.3 a	
4 Months + 2 weeksat 20 °C									
Non-treated	134.1 a	1138 a	65.7 c	15.9 a	0.38 b	77.5 a	−6.1 c	51.5 a	
1-MCP	44.0 d	9 c	75.1 b	16.1 a	0.44 a	74.5 b	−10.6 a	44.6 c	
Heat	109.9 b	1046 a	67.7 c	16.1 a	0.28 c	77.0 a	−3.0 c	51.6 a	
1-MCP+Heat	86.2 c	455 b	82.6 a	17.5 a	0.41 ab	74.2 b	−6.4 b	48.1 b	

[a] Means with the same letters within columns for each time period are not significantly different ($P = 0.05$) according to Tukey's HSD.

viability of *P. expansum* conidia in vitro and reduction of blue mold decay on 'Golden Delicious' apples by the heat treatment confirmed earlier reports on the susceptibility of this pathogen to heat and the effectiveness of the heat treatment in reducing blue mold decay on apples (Conway et al., 1999). In addition to the decay reduction by heat, the antagonist also contributed to decay reduction on heated and non-heated fruit. The 1-MCP treatment increased decay but this effect was completely diminished when the antagonist was present. 1-MCP also retarded fruit maturation in storage and during the following 2 weeks incubation at 24 °C. The heat treatment alone, and to a greater extent its combination with 1-MCP, increased populations of the antagonist in the apple wounds during the 4-d heat treatment and in storage.

A degassing period was included after the treatment with 1-MCP. Since 1-MCP treatment had no negative effect on the antagonist populations, its stimulatory effect on fruit decay results, most likely, from the direct interaction with the fruit and/or possibly from an indirect interaction with the pathogens. The enhancement by 1-MCP of both kinds of decay on 'Golden Delicious' apples, does not support the theory that less mature fruits (as measured by the maturity indices) are more resistant to fruit decay (Tucker, 1993). In strawberry, 1-MCP reduced fruit decay at low concentrations (Ku et al., 1999) but at 500 nl l^{-1} and higher concentrations it accelerated decay caused primarily by *Rhizopus stolonifer* (Jiang and Joyce, 2002; Jiang et al., 2001). The increase in decay was attributed to the reduction in phenylalanine ammonia-lyase, the key enzyme in many defense mechanisms. This may be occurring in our studies with apples as well. The mechanism of stimulating decay by 1-MCP warrants further investigation, since this compound may be used commercially on fruits in the United States in the near future.

The greatest impact of the antagonist on the reduction of bitter rot decay occurred on fruit evaluated after 4 months cold storage + 2 weeks incubation at 24 °C. The antagonist also had a higher impact in reducing decay caused by *C. acutatum*, after an incubation period of 12 h in comparison to 0 h. This means that it can effectively protect wounds from the onset of a *C. acutatum* infection for a period of time before the heat treatment, significantly reducing the overall decay. Reduction of blue mold occurred throughout the 4-month experiment and was greatly enhanced by the heat treatment. Without the heat treatment, the antagonist reduced *P. expansum* decay at either incubation period and with or without 1-MCP. The decay reduction was higher on fruit incubated for 12 h, so that the resulting decay was kept at the level of the 0 h incubation period. On heated fruit there was little decay caused by *P. expansum* and the antagonist effectively reduced the increase in decay caused by 1-MCP treatment to the already low decay level of non-1-MCP treated fruit.

Our study revealed that treatment of apples with 1-MCP increases bitter rot and blue mold decay, the two major postharvest pathogens of apple in the Eastern United States, and that combining heat with the *M. pulcherrima* T5-A2 treatments can effectively control of both of these decays even on 1-MCP treated apples.

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References

Anderson, H.W., 1956. Diseases of Fruit Crops. McGraw-Hill, New York.

Baritelle, A.L., Hyde, G.M., Fellman, J.K., Varith, J., 2001. Using 1-MCP to inhibit the inflow of ripening on impact properties of pears and apples tissue. Postharvest Biol. Technol. 23, 153–160.

Beech, F.W., 1993. Yeast in cider making. In: Rose, A.H., Harrison, J.S. (Eds.), The Yeast. Academic Press, London, pp. 169–213.

Blanpeid, G.D., Silsby, K.L., 1992. Predicting harvest date windows for apples. Cornell Coop. Ext. Bul., 221.

Bowen, J.F., Beech, F.W., 1964. The distribution of yeasts on cider apples. J. Appl. Bacteriol. 27, 333–341.

Chamberline, G., Husik, J., Subden, R.E., 1997. Freeze-desiccation survival in wild yeast in the bloom of icewine grapes. Food Res. Int. 30, 435–439.

Conway, W.S., Janisiewicz, W.J., Klein, J.D., Sams, C.E., 1999. Strategy for combining heat treatment, calcium infiltration, and biological control to reduce postharvest decay of 'Gala' apples. HortScience 34, 700–704.

Conway, W.S., Leverentz, B., Saftner, R.A., Janisiewicz, W.J., Sams, C.E., Leblanc, E., 2000. Survival and growth of *Listeria monocytogenes* on fresh-cut apple slices and its interaction with *Glomerella cingulata* and *Penicillium expansum*. Plant Dis. 84, 177–181.

Davenport, R.R., 1976. Distribution of yeast and yeast like organisms from aerial surfaces of developing apples and grapes. In: Dickinson, C.H., Preece, T.F. (Eds.), Microbiology of Aerial Plant Surfaces. Academic Press, London, pp. 325–374.

El-Ghaouth, A., Smilanick, J.L., Wilson, C.L., 2000. Enhancement of the performance of *Candida saitoana* by the addition of glycolchitosan for the control of postharvest decay of apple and citrus fruit. Postharvest Biol. Technol. 19, 103–110.

Falik, E.S., Grinberg, S., Gambourg, M., Lurie, S., 1995. Prestorage heat treatment reduces pathogenicity of *Penicillium expansum* in apple fruit. Plant Path. 45, 92–97.

Fan, X., Mattheis, J.P., 1999. Impact of 1-methylcyclopropene and methyl jasmonate on apple volatile production. J. Agr. Food Chem. 47, 2847–2853.

Fan, X., Blankenship, S.M., Mattheis, J.P., 1999. 1-Methylcyclopropene inhibits apple ripening. J. Am. Soc. Hort. Sci. 124, 690–695.

Gullino, M.L., Kuijpers, L.A.M., 1994. Social and political implications of managing plant diseases with restricted fungicides in Europe. Annu. Rev. Phytopathol. 32, 559–579.

Izumi, H., Watada, A.E., Douglas, W., 1996. Optimum O_2 and CO_2 atmospheres for storing broccoli florets at various temperatures. J. Am. Soc. Hort. Sci. 121, 127–131.

Janisiewicz, W.J., Jeffers, S.N., 1997. Efficacy of commercial formulation of two biofungicides for control of blue mold and gray mold of apples in cold storage. Crop Prot. 16, 629–633.

Janisiewicz, W.J., Korsten, L., 2002. Biological control of postharvest diseases of fruits. Annu. Rev. Phytopathol. 40, 411–441.

Janisiewicz, W.J., Marchi, A., 1992. Control of storage rots on various pear cultivars with a saprophytic strain of *Pseudomonas syringae*. Plant Dis. 76, 555–560.

Janisiewicz, W.J., Conway, W.S., Glenn, D.M., Sams, C.E., 1998. Integrating biological control and calcium treatment for controlling postharvest decay of apples. HortScience 33, 105–109.

Janisiewicz, W.J., Tworkoski, T.J., Kurtzman, C.P., 2001. Biocontrol potential of *Metchnikowia pulcherrima* strains against blue mold of apple. Phytopathology 91, 1098–1108.

Jiang, Y.M., Joyce, D.C., 2002. 1-Methylcyclopropene treatment effects on intact and fresh-cut apple. J. Hort. Sci. Biotechnol. 77, 19–21.

Jiang, Y.M., Joyce, D.C., Terry, L.A., 2001. 1-Methylcyclopropene treatment effects strawberry fruit decay. Postharvest Biol. Technol. 23, 227–232.

Ku, V.V.V., Wills, R.B.H., Ben-Yehoshua, S., 1999. 1-Methylcyclopropene can differentially affect the postharvest life of strawberries exposed to ethylene. Hort. Sci. 34, 119–120.

Leverentz, B., Janisiewicz, W.J., Conway, W.S., Saftner, R.A., Fuchs, Y., Sams, C.E., Camp, M.J., 2000. Combining yeasts or a bacterial biocontrol agent and heat treatment to reduce postharvest decay of 'Gala' apples. Postharvest Biol. Technol. 21, 87–94.

Lurie, S., 1998. Postharvest heat treatments. Postharvest Biol. Technol. 14, 257–269.

Lurie, S., Droby, S., Chalupowicz, L., Chalutz, E., 1995. Efficacy of *Candida oleophila* strain 182 in preventing *Penicillium expansum* infection of nectarine fruits. Phytoparasitica 23, 231–234.

Mari M., Carati A. 1998. Use of *Saccharomyces cerevisiae* with ethanol in the biological control of grey mould on pome fruits. Bertolini, P., Sijmons, P.C., Guerzoni, M.E., Serra, F. COST 914-COST 915 Joint Workshop—Non Conventional Methods for the Control of Postharvest Disease and Microbial Spoilage. Luxembourg, Publ. Eur. Community pp. 85–91.

McLaughlin, R.J., Wisniewski, M.E., Wilson, C.L., Chalutz, E., 1990. Effect of inoculum concentration and salt solutions on biological control of postharvest diseases of apple with *Candida* sp. Phytopathology 80, 456–461.

Mitcham B., Kader A. 1996. Methods for determining quality of fresh commodities. University of California Perishable Handling Nwslt.

Mullins, E.D., McCollum, T.G., McDonald, R.E., 2000. Concequences on ethylene metabolism of inactivating the ethylene receptor sites in diseased non-climacteric fruit. Postharvest Biol. Technol. 19, 155–164.

Pierson, F.F., Ceponis, M.J., McColloch, L.P., 1971. Market Diseases of Apple, Pear and Quinces. USGPO, Washington, DC.

Porat, R., Weiss, B., Cohen, L., Daus, A., Goren, R., Droby, S., 1999. Effects of ethylene and 1-methylcyclopropene on the postharvest qualities of 'Shamouti' oranges. Postharvest Biol. Technol. 15, 155–163.

Ragsdale, N.N., Sisler, H.D., 1994. Social and political implications of managing plant diseases with decreased availability of fungicides in the United States. Annu. Rev. Phytopathol. 32, 545–557.

Rupasinghe, H.P.V., Murr, D.P., Paliyath, G., Skog, L., 2000. Inhibitory effect of 1-MCP on ripening and superficial scald development in 'McIntosh' and 'Delicious' apples. J. Hort. Sci. Biotechnol. 75, 271–276.

Schuetz, M., Gafner, J., 1993. Analysis of yeast diversity during spontaneous and induced alcoholic fermentation. J. Appl. Bacteriol. 75, 551–558.

Smilanick, J.L., Margosan, D.A., Milkota, F., Usall, J., Michael, I., 1999. Control of citrus green mold by carbonate and biocarbonate salts and influence of commercial postharvest practices on their efficacy. Plant Dis. 83, 139–145.

Tucker, G.A., 1993. Introduction. In: Seymour, G.B., Taylor, J.E., Tucker, G.A. (Eds.), Biochemistry of Fruit Ripening. Chapman and Hall, London, pp. 1–51.

Watkins, C.B., Nock, J.F., Whitaker, B.D., 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere conditions. Postharvest Biol. Technol. 19, 17–32.

Wisniewski, M., Droby, S., Chalutz, E., Eilam, Y., 1995. Effects of Ca^{2+} and Mg^{2+} on *Botrytis cinerea* and *Penicillium expansum* in vitro and on the biocontrol activity of *Candida oleophila*. Plant Pathol. 44, 1016–1024.